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Contrasting genetic diversity of tree species in Spain: from Tertiary relicts to domestication

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Abstract

The diversity of forest ecosystems depends on the species richness, but also on the genetic variability at the intraspecific level. In this work, we investigate the contrasting patterns of genetic diversity among Spanish tree species, illustrated by means of four case studies. Combining molecular, palaeobotanical and historical information, we identify the main factors that explain the observed genetic variability of some representative taxa: (1) isolation of relict tree populations in island ecosystems of Balearic evergreen oaks and Canary Island Pine; (2) existence of glacial refugia for *Pinus sylvestris, Quercus petraea* and *Q. pyrenaica;* and (3) domestication and spread of *Ulmus minor* by Romans throughout Western Europe. These situations represent a gradient in the observed values of genetic diversity: *Pinus canariensis* and the evergreen oaks show high levels of genetic diversity in the Canary Islands and the Balearics, respectively; *P. sylvestris, Q. petraea* and *Q. pyrenaica* have also maintained high levels of genetic diversity, even under intensive, longstanding human management. Finally, *U. minor* genetic variability has been greatly affected by human activities, Dutch elm disease, and the interaction between the two. Understanding how the genetic diversity is maintained and how it is geographically distributed is basic to develop sound conservation policies and sustainable forest management strategies.

Key words: Forest ecosystems, molecular markers, human impact, phylogeography, cpDNA, microsatellites.

Resumen

La diversidad genética de las especies forestales en España: su contraste en el tiempo desde los relictos terciarios a la domesticación

La diversidad de los ecosistemas forestales depende de la riqueza en especies, pero también de la variabilidad genética a un nivel intraespecífico. En este trabajo analizamos los distintos patrones de diversidad genética de las especies arbóreas españolas, ilustrándolo con cuatro ejemplos. Combinando información molecular, paleobotánica e histórica, identificamos los principales factores que explican la variabilidad actual de varios taxones representativos: (1) el aislamiento en ecosistemas insulares de poblaciones arbóreas relictas de robles esclerófilos baleares y pino canario; (2) la existencia de refugios glaciares para *Pinus sylvestris, Quercus petraea* y *Q. pyrenaica;* y (3) la domesticación y expansión de *Ulmus minor* por los romanos en Europa Occidental. Estas situaciones representan un gradiente en los valores observados de diversidad genética: *Pinus canariensis* y los robles esclerófilos muestran altos niveles de diversidad genética en las Islas Canarias y las Baleares respectivamente; *Pinus sylvestris, Quercus petraea* y *Q. pyrenaica* también han mantenido elevados niveles de diversidad genética a pesar de haber sufrido un intensivo y prolongado manejo por parte del hombre. Finalmente, la diversidad genética de *Ulmus minor* se ha visto grandemente afectada por el impacto humano, la enfermedad de la grafiosis y la interacción entre los dos factores. Comprender cómo se mantiene la diversidad

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genética y su distribución geográfica es básico para establecer sólidas medidas de conservación y estrategias de manejo forestal sostenibles.

Palabras clave: Ecosistemas forestales, marcadores moleculares, impacto humano, filogeografía, cpDNA, microsatélites.

Introduction

Since Neolithic times, the interaction between the human being and the forests has been essential for the development of civilizations, as tree species provide a renewable supply of valuable basic materials and indirect environmental benefits. Today, after thousands of years of intense human pressure on forests, the necessity of preserving the remaining woodlands from fragmentation, desertification and global climatic change is evident (UN Conference of Environment and Development, Río de Janeiro, 1992). Therefore, it is necessary to investigate the evolutionary dynamics of forest ecosystems in order to identify the major factors that affect their adaptability to environmental changes.

A method of monitoring the changes in ecosystems through time is the study of genetic diversity of the species that constitute them. The level of genetic variation within and among the populations of a species determines its adaptive potential to fast environmental shifts. Moreover, molecular biology is providing fast and simple tools for the inference of otherwise unobservable demographic and evolutionary processes (Haig, 1998) and for understanding how historical events (range expansion/reduction and fragmentation) have shaped present-day demographic and genetic structure (Moritz, 1995). Most molecular markers give us estimations of neutral molecular diversity, which can be used to make inferences about evolutionary factors others than selection, such as migration, genetic drift and mutation. The use of different types of molecular markers (showing contrasting modes of inheritance, ploidy levels or mutation rates) and the comparison of observed patterns between sets of taxa can provide useful information about the interplay between genetic factors, demography, population history, and life history traits.

Forest tree species are the group of plants with the highest degree of control over the environment and they have a predominant ecological role in the terrestrial biosphere. Thus, they constitute the basis that sustains the rest of the components of forest ecosystems (Terradas, 2001). Genetic diversity of forest tree species can be influenced by several intrinsic and extrinsic factors. The effective population size of the forest communities is affected in first instance by its spatial geometry. While some species are forced to create small isolated patches (e.g. *Ulmus* sp. and *Populus* sp. associated to water courses), some others can conform stable forests of thousands of hectares (e.g. *Pinus* sp. and *Quercus* sp.). Spatial distribution is mainly regulated by the ecological constraints of each species (e.g. requirements of soil, moisture or temperature) and by the interaction with other components of the ecosystem (e.g. tolerance to shade, resistance to plagues, etc.).

On the other hand, reproductive strategies of these species have also a strong effect in the genetic structure of the populations. Predominance of sexual or asexual reproduction, self-incompatibility mechanisms, anemophily and absence of clear reproductive barriers between species of the same genera will define the genetic composition of the progenies replacing the current members of the populations, as well as the ability of the species to colonize new areas. We have to keep in mind that life cycles of trees are long and, therefore, their long-term survival is not only dependant on the reproductive success in a single year, but on the endurance of changing environmental pressures and the contribution of new individuals to the next generation in a long temporal frame. Therefore, tree populations and forest ecosystems are expected to show a certain degree of resilience and long term stability.

However, stability of these ecosystems is frequently disturbed by perturbations of abiotic and biotic origin. In the long term, abiotic perturbations correspond to palaeogeographical events produced by continental displacements and periodical climatic oscillations, mainly Quaternary glacial advances and retreats. In particular, fossil pollen evidences and molecular techniques have recognized the southern Mediterranean peninsulas as glacial refugia from where most temperate tree species recolonised northern Europe after the Last Glacial Maximum (Huntley & Birks, 1983; Hewitt, 1996; Taberlet *et al.*, 1998; Petit *et al.*, 1997).

Finally, an essential factor affecting the genetic diversity of tree populations is the intense human activity during the last 5,000 years, which has modelled the landscape (Thirgood, 1981) and acted as a catalyst of the modification of forests by direct management or by favouring fragmentation or introduction of alien organisms (plagues and diseases, invasive species, etc.), among other factors.

When we examine the tree species present in the Spanish ecosystems (Iberian Peninsula, Balearic and Canary Islands) we find representatives of some of the most important genera in the Palaeartic region, frequently merging together. Genus *Pinus* contributes with seven species *(P. sylvestris, P. pinaster, P. halepensis, P. nigra, P. uncinata, P. pinea* and *P. canariensis)*. Among angiosperms the complex *Quercus* shows 10 species and several intermediate forms *(Q. suber, Q. ilex, Q. coccifera, Q. robur, Q. petraea, Q. pyrenaica, Q. faginea, Q. humilis, Q. canariensis* and *Q. lusitanica)*, genus *Ulmus* three species *(U. minor, U. laevis* and *U. glabra)* and genus *Populus* another three species *(P. nigra, P. alba* and *P. tremula)*. Some other genera with importance but with a single representative in Spain are *Fagus (F. sylvatica)*, *Castanea (C. sativa)* or *Olea (O. europaea)*.

In this paper, we present an overview of the genetic diversity levels of representative species of the main tree genera distributed through the Iberian Peninsula and the Balearic and Canary Islands. Citing some examples, we will point out the differences in genetic diversity levels among species and we will relate them to the factors that have led to the current genetic structure of the populations, such as macrogeological events, climatic fluctuations and the more recent human activities.

Genetic diversity of island tree populations

Islands present both diversity and stability paradox. They are often highly species-poor but have considerable biological interest in terms of endemic genera and taxonomically isolated groups. They appear to be stable, as in some cases island-inhabiting organisms have persisted for a long time, and, having an oceanic climate, extreme climate events may be comparatively rare (Cronk, 1997). Since they are isolated, evolutionary processes work at different rates, with little or no gene flow to dilute the effects of selection and mutation. At the same time, isolation makes islands more vulnerable to habitat change and extinction.

Because island populations are often isolated from mainland populations, they usually diverge over time from each other due to genetic drift, changed selection pressures, or both (Johnson, 2000). The magnitude of this divergence will mainly depend on three factors: extinction (how often accumulated divergence is erased by extinction), colonization (how often new island populations are established) and migration (how often new migrants from the mainland population contribute to the island gene pool), and they can ultimately lead to speciation (Adler, 1992).

Two particular cases within Spanish ecosystems are those of the Balearic and Canary Islands. In addition to the latitudinal differences between both archipelagos, they belong to different biogeographic regions and have had different geological histories. While the Canary Islands are oceanic islands of volcanic origin, the Balearics are continental islands, that is, they were once connected to the mainland. Besides, both archipielagos have been subjected to different degrees of human impact, from the historical intense trade in the Mediterranean to the relative isolation of the Canary Islands. These differences are essential to understand the biological patterns of Canarian and Balearic tree species.

Taxa inhabiting the Balearics are the product of vicariance and long distance dispersal events. The main constraint in the current distribution and phylogeographic structure of Balearic taxa is the geological history of the archipelago. Indeed, the Balearics' geological history is complicated, as they lie in a very unstable area that has suffered the effects of continental displacement (De Jong, 1998), desiccation of the Mediterranean sea during the Messinian (Krijgsman *et al.,* 1999) and marine transgressions (Colom, 1978) along the last 30 million years. As a consequence, along with taxa introduced by humans, we can find groups of endemic plants in the Balearics that are closely related to those from Catalonia-Provence, Baetic-Riffean areas or even Tyrrhenian areas (Colom, 1978).

The evergreen oak complex *(Quercus suber, Q. ilex* and *Q. coccifera)* is a good example of the different degrees of relationships with mainland populations surrounding the Balearics. By means of chloroplast DNA (cpDNA) analysis of populations from the

Balearics and the surrounding regions (southeastern Spain, Catalonia, Provence, Sardinia, Corsica, Sicily and northern Africa), it is possible to identify the sources from which the balearic populations of evergreen oaks originated, because cpDNA is maternally inherited in angiosperms, and therefore, it reflects the movement of seeds. Actually, *Q. coccifera* and *Q. suber* show a mixture of Iberian and Tyrrhenian lineages, while *Q. ilex* populations belong to a single lineage undoubtedly related to eastern Iberian populations (Jiménez *et al.*, 2004; Lopez-de-Heredia *et al.*, 2005). A similar lineage admixture occurs in the Balearic *Pinus pinaster,* which shows a low effective population size (only a few individuals). The cpSSR analysis of two of these individuals showed a common haplotype in both Tyrrhenian and Iberian regions, while nuclear microsatellites (nSSRs) revealed the presence of an allele which is very frequent in Corsica and Provence (S.C. González-Martínez, pers. comm.).

The occurrence of three lineages of evergreen oaks in the Balearics reveals them as a contact area that has probably sustained the species since stadials prior to the Pliocene marine transgression that separated the islands from the mainland, though posterior long distance dispersal from close areas cannot be discarded (López de Heredia *et al.*, 2005). The result is a high level of total diversity for the three species. Balearic *Q. suber* populations have a total diversity ($h_{T(suberBal)}$ = 0.861; *SD* = 0.036) higher than the overall diversity across the whole range of the species ($h_{T(suberTot)}$ = 0.655; *SD* = 0.065), but their intrapopulation diversity is low ($h_{S(suberBal)}$ = 0.067; *SD* = 0.022). In *Q. coccifera,* however, there is no intrapopulation diversity ($h_{S(cocciferaBal)}$ = 0), but total diversity is moderate ($h_{T(cocciferaBal)}$ = 0.667; *SD* = 0.139). Total diversity in *Q. ilex* is high ($h_{T(ilexBal)}$ = 0.705; *SD* = 0.048), but not as much as that of *Q. suber,* because a single lineage is present in the former, and there are two lineages in the latter. Finally, there is a high intrapopulation diversity in *Q. ilex* ($h_{S(ilexBal)}$ = 0.303; *SD* = 0.060), similar to the intrapopulation diversity observed in the Iberian Peninsula forests ($h_{S(ilexTot)}$ = 0.2156; *SD* = 0.025).

The haplotypic composition of the evergreen oaks in the Balearics is shown in Figure 1. It is remarkable that even some marginal populations of very low effective population size (e.g. *Q. ilex* from Ibiza, *Q. suber* from Majorca) present as many as four different cpDNA haplotypes. Indeed, areas like southern Majorca are the most suitable for the development of *Q. ilex* forests, surely more extensive in the past (Rivas-Martínez and Costa, 1987; Rivas-Martínez *et al.*, 1992). Unfortunately, these areas are also preferred for

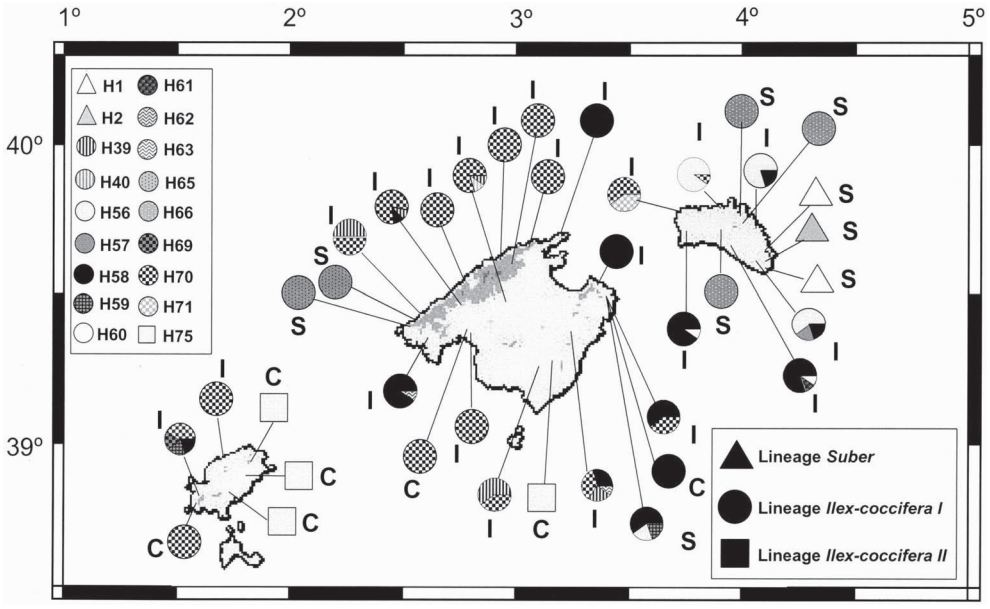

Figure 1. cpDNA haplotypic diversity and lineages in evergreen oaks populations from the Balearic Islands. I: *Quercus ilex,* C: *Quercus coccifera,* S: *Quercus suber.* Modified from Lopez-de-Heredia *et al.* (2005).

agriculture, and anthropic pressures on the *Q. ilex* forests have caused their fragmentation and reduction, although the genetic diversity has somehow been maintained. On the other hand, the Sierra de Tramuntana mountain range is virtually occupied by a single private haplotype that only appears in the *Q. ilex-coccifera* populations from the Balearic Islands (Fig. 1). Haplotype sharing with eastern Iberia could be the result of long distance colonization events, though these are not likely in heavy-seeded species such as *Quercus,* since the acorns die when they are dehydrated under 40% (Montero and Cañellas, 1999). Therefore, the contribution from long-distant areas must have been very limited.

In contrast to the Balearics, the only way to colonize the volcanic Canary Islands is through rare long-distance seed dispersal events from the mainland. However, this does not seem to be a limitation for Canarian ecosystems, which show a high number of endemic plants, approximately 570 species (Francisco-Ortega *et al.,* 2000). An example of endemic tree is the Canary Island pine *(Pinus canariensis* C. Sm.), that inhabits the westernmost islands (Gran Canaria, Tenerife, La Palma, El Hierro and La Gomera), and that went extinct in the easternmost islands in historical times. Actually, *P. canariensis* is a survivor of a subtropical mountain pine type already present in the Tertiary in an ancient Mediterranean evolutionary center (Klaus, 1989; Gil *et al.,* 2002).

The overall genetic diversity of *P. canariensis* is concentrated in few populations from the islands that inhabits. Recent genetic studies have shown evidences of colonization from eastern to western islands, coinciding broadly with their geological date of emergence (Gómez *et al.,* 2002). Nowadays, *P. canariensis* is not living in Fuerteventura and Lanzarote, but these islands could have been migration pathways to the rest of the Canary Islands from a single continental source located close to the Mediterranean Basin. Two independent migration pathways have been proposed, one from northwestern Tenerife to La Palma and the other from southeastern Tenerife to El Hierro through La Gomera (Gómez *et al.*, 2002). However, the latter route is not well supported by a recent cpDNA study, in which La Gomera pines appear strongly genetically differentiated from those occupying La Palma-El Hierro (Vaxevanidou *et al.,* submitted). As opposed to heavy seed species (i.e. *Quercus)*, long distance dispersal of *P. canariensis* is very likely facilitated by its seed traits (adnate seed wings). Therefore, a rapid colonization of the Canary Islands may have been possible, becoming the refuge for the species after climatic changes occurred in the Mediterranean region during the Pliocene.

Recent studies have shown a high haplotypic diversity at cpDNA microsatellites for *P. canariensis* (Vaxevanidou *et al.,* 2004). In a survey of 17 populations, 69 different haplotypes were identified (Fig. 2). While there is only

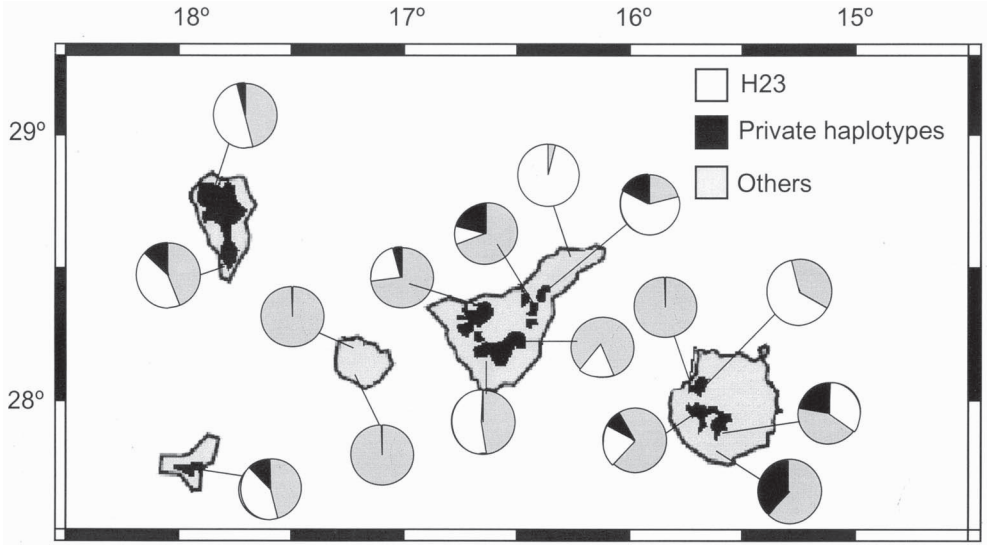

Figure 2. cpDNA Haplotypic diversity of *Pinus canariensis* modified from Vaxevanidou *et al.* (2004). Others indicate those present in at least two populations.

one widespread cpDNA haplotype, from which different geographically structured chloroplast lineages were probably derived, it is also noteworthy the presence of many private haplotypes. Along with the high haplotypic differentiation among populations, F_{ST} = 0.27, it can be interpreted as a generalized high degree of population isolation. On the other hand, the populations usually show high intrapopulation diversity, especially in marginal populations like Arguineguin (southern Gran Canaria), where 24 different haplotypes were observed in a sample of 30 individuals (Vaxevanidou *et al.,* 2004). This population is a relict isolate surviving under extremely dry conditions in a predominantly deforested landscape (Voggenreiter, 1976). Again, like in the case of *Q. ilex* in southern Majorca, the effect of the large-scale deforestation in the last centuries is obvious (Parsons, 1981). The Arguineguin population presently comprises less than a hundred trees, which seem to be the remnant of a larger population severely reduced by human intervention and a climatic change towards drier conditions.

Summarizing, molecular markers have revealed both the Balearic and Canary Islands as genetic diversity reservoirs for several tree species. However, because of insularity, they are more prone to physical or human mediated perturbations. Conservation policies should take into account the role of insular populations in the maintenance of the genetic diversity of tree species.

Genetic diversity of white oaks in the Iberian Peninsula

By contrast to island populations, forest trees occupying mainland areas can be expected to have undergone range shifts and geographic movements on a larger scale in response to climatic changes. The use of molecular markers to analyse the genetic variation and its geographic distribution in plant genomes with contrasting modes of inheritance (uniparental chloroplast DNA and biparental nuclear DNA) is shedding light on the colonisation patterns of open habitats and northern European regions by tree species after the last glacial age (Hewitt, 1996; Taberlet *et al.*, 1998; Petit *et al.*, 1997). The available molecular information suggests that colonisation by most temperate tree species took place from southern refugia through a combination of population diffusion and long-distance seed dispersal, and that extensive pollen flow has subsequently homogenised the genetic structure across colonised regions *(e.g.*, Ennos, 1994; McCauley, 1995; Demesure *et al.,* 1996; El Mousadik and Petit, 1996; but see Palmé *et al.*, 2003 and Soranzo *et al.,* 2000 for evidences of more northerly refugia).

White oak species (genus *Quercus,* section *Lepidobalanus)* represent a very interesting case study on the inference of recolonisation patterns from neutral molecular variation, due to their contrasting levels of pollen vs seed flow and to the lack of clear reproductive barriers among the species of the section. Some studies focussing on the flowering and fruiting patterns of these species have shown that the effective number of trees contributing to the next generation as females is much lower than the effective paternal population size (Demesure *et al.,* 1996; Dow and Ashley, 1996). Moreover, systematic hybridisation and introgression between oaks is essential to explain the geographic structuring of different maternal lineages, which are frequently shared among sympatric species of the genus all over Europe (Dumolin-Lapègue *et al.*, 1997). In fact, prolonged opportunities for hybridisation in refuge areas with small effective population sizes are expected to enhance the occurrence of cpDNA sharing through pollination among interfertile species (Dumolin-Lapègue *et al.*, 1999). Therefore, introgressive hybridisation must be taken into account when interpreting the phylogeographic pattern of the species.

The white oak section (subgenus *Quercus)* comprises six species in the Iberian Peninsula, according to Franco (1990) –*Q. robur, Q. petraea, Q. pyrenaica, Q. faginea, Q. canariensis, Q. humilis*–, along with a plethora of hybrid forms. A molecular survey extending over the European range of these species has found 14 cpDNA haplotypes belonging to 4 lineages (Petit *et al.*, 2002). Seven of these haplotypes are exclusive to the Iberian Peninsula (Fig. 3).

Traditionally the existence of primaria refugia for white oaks was only considered in southern Iberia (Brewer *et al.,* 2002), and fossil pollen records did not evidence them at higher latitudes. However the existence of multiple refugia for other species in the Iberian Peninsula, including central and northern locations, has been pointed out recently (Gómez and Lunt, 2004) and new palynological (Carrión *et al.*,

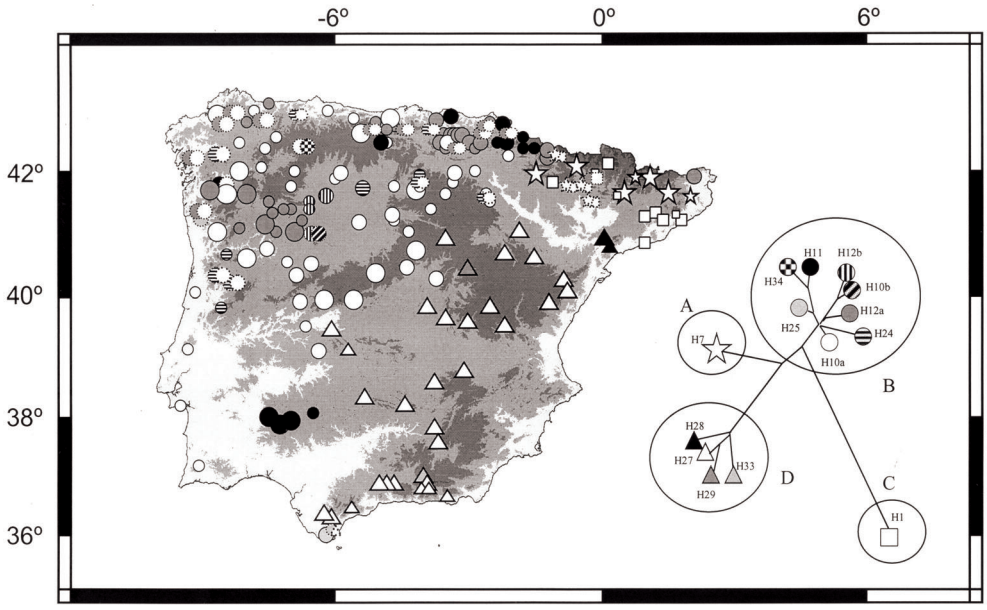

Figure 3. Distribution of cpDNA haplotypes in Iberian white oaks and maximum parsimony tree of haplotypes. Lineages are also shown. Dotted symbols correspond to sympatric populations. Modified from Olalde *et al.* (2002).

2003) and molecular (our unpublished data) information in evergreen oaks support this hypothesis. As new pollen information is gained, a reinterpretation of geographic location of primaria refugia for white oaks might be necessary. Basal mountain valleys and deep gorges are the best candidates to host temperate species during harsh climatic periods (Costa Tenorio *et al.,* 1990). In fact, altitudinal population dynamics, rather than latitudinal factors, seems to have played a most important role in the recent history of vegetation in the Iberian Peninsula (Gates, 1993). The geographic structuring of the different maternal lineages into well-defined biogeographic regions suggests that this structure might have been present before the last ice-age (Olalde *et al.*, 2002).

Following Hewitt (1996)'s predictions and Newton *et al.* (1999)'s study, higher haplotypic diversity is expected in glacial refuge areas than in northern locations of a species. The level of subdivision for cpDNA polymorphisms in the Iberian white oaks populations is among the highest found for angiosperms. There are high levels of total genetic diversity (ranging from h_T = 0.600; *SD* = 0.064 to h_T = 0.804; *SD* = 0.056 among different species), and low intrapopulation diversity (from h_S = 0.030; *SD* = 0.021 to h_S = 0.205; *SD* = 0.048) (Olalde *et al.*, 2002), suggesting a colonization process based on long-distance acorn dispersal from separate refugial locations.

Diversity levels of Iberian white oaks are similar to those found in other European countries (Petit *et al.*, 2002). Specifically, total diversity rates were high *(Q. faginea* h_T = 0.783; *SD* = 0.037; *Q. petraea* h_T = 0.804; *SD* = 0.056; *Q. humilis* h_T = 0.679; *SD* = 0.043; *Q. pyrenaica* h_T = 0.600; *SD* = 0.064; *Q. robur* h_T = 0.666; *SD* = 0.038), while intrapopulation diversity rates were low *(Q. faginea* h_S = 0.031; *SD* = 0.018; *Q. petraea* h_S = 0.110; *SD* = 0.073; *Q. humilis* h_S = 0.083; *SD* = 0.057; *Q. pyrenaica* h_S = 0.030; *SD* = 0.021; *Q. robur* h_S = 0.205; *SD* = 0.048). Total diversity levels are similar in pure and mixed populations (Pure h_T = 0.792; *SD* = 0.020; Mixed h_T = 0.791; *SD* = 0.030).

Contemporary demographic processes are also shaping the diversity pattern of the different species. Ecological factors and adaptation to environmental changes module the presence and abundance of different taxa in different biogeographic regions. Oak hybridisation, which is evident in refuge areas, might have played a very important role in the genetic homogenisation of taxa, with subsequent adaptation to local environment being responsible for the predominance of the better adapted ecotypes.

A potential preglacial hybrid system of white oaks is that of *Q. petraea* and *Q. pyrenaica* in La Sierra Norte (Central System range, Madrid), where the combination of Atlantic and Mediterranean climatic factors modules the presence of flora elements from both ecoclimatic regions. Both species have different ecological requirements and distinct adaptation to local environments. *Quercus petraea* populations represent the southernmost margin of its range in Europe, growing under limiting ecological conditions (water deficit during summer strongly affects growth and survival). In contrast, *Quercus pyrenaica* is widely distributed in the region, and very well adapted to Oromediterranean ecological conditions. *Q. pyrenaica* is the dominant tree species of the montane belt slopes of the Central System Mountain Range, due to phenological adaptation and its root and stem sprouting ability, which confers selective advantage to ecological perturbations such as forest fires.

In this area, both *Q. petraea* and *Q. pyrenaica,* are fixed for cpDNA haplotype *H10a* (extended nomenclature after Dumolin-Lapègue *et al.*, 1997; our unpublished results), the most common haplotype of the Iberian *B* cpDNA lineage (Olalde *et al.,* 2002). Whether coincident postglacial recolonisation routes from the same refugia or posterior contact of different species migration routes are responsible for the existence of a fixed haplotype in La Sierra Norte remains uncertain.

The contrasting ecological adaptations of the species do not result in differences in neutral nuclear diversity levels, as estimated by nSSR (H_{eTot} = 0.888; *SD* = 0.052 for *Q. petraea* and H_{eTot} = 0.869; *SD* = 0.090 for *Q. pyrenaica)*. Heterozygosity is high for both species, within the range of other European oak populations (Mariette *et al.,* 2002). On the other hand, differentiation among populations is low but significant (p = 0.000) for both species (F_{ST} = 0.011-0.029 for *Q. petraea* and F_{ST} = 0.005-0.028 for *Q. pyrenaica)*. Similarly low levels of genetic structuring have been found in other regional-scale studies in oaks, generally related to high pollen mediated gene flow (Dow and Ashley 1998; Streiff *et al.*, 1999). However, in La Sierra Norte, *Q. petraea* populations are genetically more differentiated than those of *Q. pyrenaica* over the same scale, probably due to contrasting population sizes, markedly lower in *Q. petraea.*

Parentage analyses conducted on established offspring in one of the oak populations of La Sierra Norte have shown similar patterns of effective gene dispersal in the two species, despite contrasting population sizes (Valbuena-Carabaña *et al.,* in press). Immigration rates were similar, with a gametic flow from outside the plot (ca 13 ha) of ~38% for *Q. petraea* and ~34% for *Q. pyrenaica.* Therefore, the scattered distribution of *Q. petraea* populations in the area does not result in higher genetic isolation, as compared to *Q. pyrenaica,* whose populations are embedded within the core of its distribution. Nevertheless, at the within-population level, specific disparities in gene movement are observable: restriction in dispersal distances reach different values for *Q. petraea* (75 m) and *Q. prenaica* (25 m). These contrasting patterns might arise from differences in dispersal ability, such as those arising from variation in acorn size, which makes *Q. pyrenaica* seed movement more restricted than *Q. petraea*'s (3.26 g; *SD* = 0.67 for *Q. pyrenaica,* and 2.14 g; *SD* = 0.68 for *Q. petraea,* Valbuena-Carabaña *et al.*, in press). Secondary-dispersal by animals seems also to be a relevant factor affecting recruitment. As a consequence, not only acorn size and weight, but also disperser preferences due to acorn palatability, might account for specific dispersal patterns (Bossema, 1979), especially because masting is a common feature affecting recruitment in the two species.

Results of the parentage analysis reflect that limiting ecological conditions do not constrict the recruitment in *Q. petraea.* The species seems to be well adapted to the marginal conditions of the area, which do not have significant effects on gene flow or diversity levels of the species. Therefore, anemophily seems to be an effective strategy in maintaining high genetic diversity and connectivity subsequently to population fragmentation. This area has been intensively managed historically (Gil *et al.*, 1999), indeed oak forests were transformed into open woodlands to favour pasture and acorn production. Low population density could have maintained abundant pollen flow, leading to high levels of genetic diversity.

Due to its root sprouting capability, *Q. pyrenaica* has also been managed as a coppice system, providing excellent conditions for charcoal production, logging and cattle grazing uses. Whether the vegetative recruitment of *Q. pyrenaica* under this sort of management may lead to genetic erosion is an open issue to be considered in future studies, since preliminary data show high rates of heterozygosity (as high as in open woodlands) and only sparse presence of

clonal clumps. High resolution molecular markers, such as our battery of nSSR, will be helpful in order to investigate the genetic consequences of coppicing in *Q. pyrenaica* populations.

Evolutionary dynamics of montane populations of Scots Pine

Scots Pine *(Pinus sylvestris* L.) populations in the Iberian Peninsula constitute an interesting system to illustrate the role of species biology, population history, and regional ecology in the evolutionary dynamics of our mountain forests. They are considered Tertiary relicts that have persisted through Quaternary glaciations within this region, the southernmost limit of the species distribution (Willis *et al.*, 1998). They are believed to have endured several glacial cycles somewhat isolated from the widespread northern populations, developing unique genetic characteristics at nuclear loci (Prus-Glowacki *et al.*, 2003), mitochondrial DNA regions (Soranzo *et al.*, 2000) and quantitative traits (Alía *et al.*, 2001). And interestingly, while they presently occupy what represents a warm, marginal area for the species, with fragmented populations sheltering at high elevation on different mountain chains, they maintain neutral genetic diversity levels higher than those of typically Mediterranean Iberian pines. Several studies using a common set of chloroplast microsatellite markers (cpSSR) have shown that the genetic diversity differences with *P. pinea* and *P. halepensis,* the most thermophilic species of the genus in the region, are substantial: the population haplotypic diversity for Scots pine is significantly much larger than those of *P. pinea* and *P. halepensis,* while *Pinus pinaster,* a species with a high ecological plasticity, shows diversity values only slightly smaller than those of *P. sylvestris* (our unpublished results; Vendramin, pers. comm).

Why Scots pine, a species adapted to cold and wet conditions, shows higher genetic diversity values in the Iberian Peninsula than other members of the genus that could be expected to be better adapted to the Mediterranean habitat? The answer lies probably on the erroneous assumptions that the present ecological setting is representative of those extreme conditions that have shaped the observed neutral genetic variation of these species in the long term. If Scots pine populations in Spain had conformed to present-day «montane island» pattern for a long time, we would expect to find substantial genetic differentiation among populations (Oline *et al.*, 2000), as well as greater genetic similarity among populations on the same mountain than among populations from different ranges.

A fine-scale molecular survey in the Northern Meseta and peripheral mountain chains has shown that this is not the case. Robledo-Arnuncio *et al.* (2005) found that Scots pine populations in the region exhibit a rather low level of genetic differentiation (ϕ_{ST} = 0.031, p = 0.010) and, besides, that this is mainly due to divergence among populations within the same mountain block (ϕ_{SC} = 0.021, p = 0.012). In fact, the genealogical relationships among populations suggested that Scots pine isolates growing on disjoint mountain blocks, but on slopes flowing to the same basin, were genetically closer than populations growing on different slopes of the same mountain chain (Fig. 4). Valleys seem to have served as genetic corridors for historical gene exchange among presently distant populations.

This hypothesis is consistent with the strong palaeobotanical evidence suggesting that *P. sylvestris* found a suitable habitat in the inner plateaus during

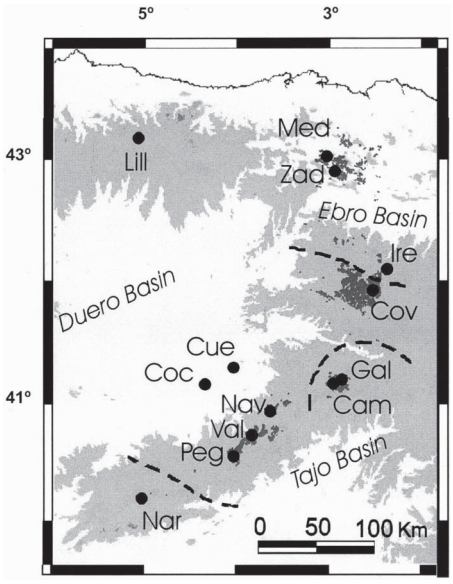

Figure 4. Distribution of *Pinus sylvestris* L. in the Northern Meseta (Spain) and location of the thirteen populations (black dots) sampled for the chloroplast-microsatellite genetic study. Dotted lines separate genetically differentiated areas, as suggested by the genealogical relationships among populations. Codes and details about the populations can be found in Robledo-Arnuncio *et al.* (2005).

cold stages (Franco-Múgica *et al.*, 2001), establishing widespread woodlands for long periods. Despite moderate periodic interstadial fragmentation episodes (such as present-day's), Scots pine adaptation to low temperatures, coupled with the swift response to climatic pulses allowed by short-distance altitudinal migrations, would have provided for the long-term maintenance of large effective population sizes, resulting in the observed high within-population and low among-population diversity at neutral markers. By contrast, more termophilic low-elevation taxa, such as *P. pinea* and *P. halepensis,* would have been unable to endure the harsh continental climate of inner regions during cold stages, suffering further fragmentation and dramatic range contraction into small coastal refugia, which may have led to repeated bottlenecks and subsequent gene diversity loss (Morgante *et al.*, 1998). Another explanation for the relatively low genetic diversity of *P. halepensis* could be a recent migration of the species into the Iberian Peninsula from the Eastern Mediterranean: the genetic erosion caused by this long-distance movement might have resulted in reduced genetic diversity relative to other pine species with an earlier presence in the Iberian Peninsula (Gómez *et al.* 2005).

Although Scots pine neutral genetic diversity level appears to be resilient to long-term demographic dynamics caused by climatic changes, we can wonder whether contemporary anthropogenic disturbances may be producing a significant impact on the natural evolutionary processes of the species. This question becomes especially relevant in those populations where tree harvesting is an important economic activity. There is evidence that population size and density reductions may occasionally lead to increased selfing and correlated paternity in plant populations (Kennington and James 1997; Paschke *et al.*, 2002), which across generations can result in inbreeding depression, genetic erosion, and fitness loss. However, an investigation of the genetic effects of two natural regeneration methods (shelterwood and group selection cutting) of *P. sylvestris* in Valsaín and Navafría (Guadarrama Chain) showed that pollen movement within large monospecific Scots pine populations is extensive enough to make effective pollination rather insensitive to a wide range of densities (80-300 trees/ha), including those under which natural regeneration is generally achieved (Robledo-Arnuncio *et al.*, 2004a). This result suggests that, from the pollination point of view, reasonable harvesting of Scots pine forests by means of natural regeneration methods is compatible with genetic conservation objectives.

But the reproductive biology of such a resilient species as *P. sylvestris* may indeed be greatly affected by human-mediated demographic attrition. An interesting example is given by the Coca population, a relict stand comprising less than fifty Scots pine trees, growing on the sandy soils of the inner Northern Meseta (Segovia), 30 km far away from the closest population of the species (coded as Coc in Fig. 4). This stand represents a remnant fragment of the more widespread distribution of the species that occupied the plateau until the Mid-Late Holocene period. An historical document suggests that its size was still economically significant in the 16th century (cited in Álvarez and Allué, 1997). Increasing human pressure, land-use change, and the lowering of the water table in the area caused by irrigation, have produced the severe size reduction leading to current numbers. The remaining trees of this typically outcrossed species show an average selfing rate of 0.25 (some individuals as high as 0.90), eight-fold higher than the average in large Scots pine populations in the region (Robledo-Arnuncio *et al.* 2004b). The correlated paternity rate is also being severely affected: maternal sibships from Coca seed trees contain on average 20% of full-sibs, a value 100-fold higher than that found in the large populations of the species.

Although the most pressing problem in Coca is to recover the phreatic water supply that would allow the natural regeneration of the population (presently inexistent), subsequent management activities should ensure the conditions to avoid the long-term deleterious effects of such a high levels of selfing and correlated mating. Since the severe size reduction of the population is likely to have taken place over the last few decades, an increase in selection against recessive lethals has probably not yet begun, and therefore the next generations can be expected to suffer severe inbreeding depression. Among conservation activities, increasing population size and density by planting local non-inbred seedlings would be an advisable initial step. The use of genetic markers could assist in designing controlled crosses that minimize inbreeding of the used planting material (Fernández *et al.* 2003) and, in a later stage, in monitoring the mating system and genetic dynamics of this singular remnant.

Field elm, a case of domestication of a forest tree species

Field elm *(Ulmus minor* Mill.) is a riparian tree species widely distributed across Europe, although its adaptation to summer drought makes it more common in southern countries (Richens, 1983). River banks, where natural populations are found, are also the most suitable places for agriculture and grazing, and field elm soon became part of human-altered landscapes (Gil and García Nieto, 1990). The quality of fodder obtained from elm leaves, the use of its wood for different purposes, and its ability to regenerate asexually by root suckers, were soon recognised by humans (Gil *et al.*, 2003; Heybroek, 2003). As a result, this tree has been commonly planted in Spain and other countries even far from its natural habitat (cities, parks, avenues, etc.). It is likely that the felling of natural elm-groves for the cultivation of the land in the Iberian Peninsula took place in restricted regions until the Roman period (Gil and García Nieto, 1990). Then, large-scale cultivation in those areas where elm populations occurred resulted in lost of genotypes, with subsequent pressure on the remaining trees by livestock favouring those individuals that were able to produce more root suckers. At the same time, the Romans also spread the cultivation of elm for its fodder and, above all, for its use as living props for the vines (Fuentes-Utrilla *et al.,* 2004).

The use of trees for vine training was probably developed in the Neolithic at the same time as viticulture, when the growth of wild vines over trees was imitated by the early vine-growers. There are evidences of the use of trees for vine training during the Assyrian Empire in the 7th Century BC and in the Ancient Greece since 6th Century BC (Fuentes-Utrilla *et al.*, 2004). Already in the 3rd Century BC, elm appears related to this purpose in Theocritus' Idylls (Hunter, 1999). With the Roman domination, a change in the conception of agriculture took place, from a subsistence type based on grain towards a commercial system in which wine and olives played a significant role (Brehaud, 1933). Elms and poplars were the recommended trees for plantations, because they were good props for vines, and they provided fodder for sheep and oxen, poles for fences and firewood (Varro, *De Re* Rustica; Hooper and Ash, 1934)). The process of selection of the best supporting trees for the vines reached its maximum during the 1st century AD. The agronomist Columella, who was a great defender of a capitalistic conception of agriculture, based on large farms and slave labour (Martin, 1971), emphasized that vineyards were by far the most profitable crop due to the high incomes obtained from wine, and also indicated that in order to obtain good quality wines, the vines should be grown married to trees, in particular to poplars and elms *(De Re Rustica;* Foster and Heffner, 1954). Interestingly, this author recognized two types of elms, the *native* and the *Gallic* or *Atinian.* Columella described the latter as faster-growing and having a denser canopy than the native, and explained that this elm did not set seeds, so it was only reproduced by root suckers. He recommended the Atinian elm for plantation in vineyards because its fodder was the most appreciated by oxen.

Molecular markers have recently demonstrated the transport and spread of Italian elms, including the Atinian, in the Iberian Peninsula and England (Gil *et al.*, 2004). Roman domestication of elms for vine training involved a reduction in the number of genotypes that were used which were afterward asexually propagated. Yet to what extent have Roman transport and cultivation of elms changed the genetic diversity of the Spanish elm populations? That is, were Iberian elms more diverse before Roman cultivation? The answer is not straightforward because it depends on which component of the genome (nuclear vs. chloroplast DNA) we consider.

Using chloroplast DNA (cpDNA) markers, Gil *et al.* (2004) were able to identify four mayor lineages in Europe, two of them in the Iberian Peninsula (lineages C and D; Fig. 5). Lineage D, present in *U. minor* and also in *U. glabra,* consists of six haplotypes that remained in refuge areas during the last glacial period, whereas the six haplotypes belonging to lineage C (only in *U. minor)* come from those elms introduced from Italy by the Romans. Both lineages are widely distributed across the Peninsula, which in the case of lineage C can give us an idea of the extent that Roman viticulture had in our country. In terms of number of haplotypes present in Spain before and after Roman viticulture, the absolute genetic diversity estimated by cpDNA was doubled by the human activity (six native plus six introduced). On the other hand, rare native haplotypes of lineage D could have probably been lost when natural elm populations were clear-cut for cultivation. However, lineage D also shows little genetic diversity in *U. glabra* (2 haplotypes, one of

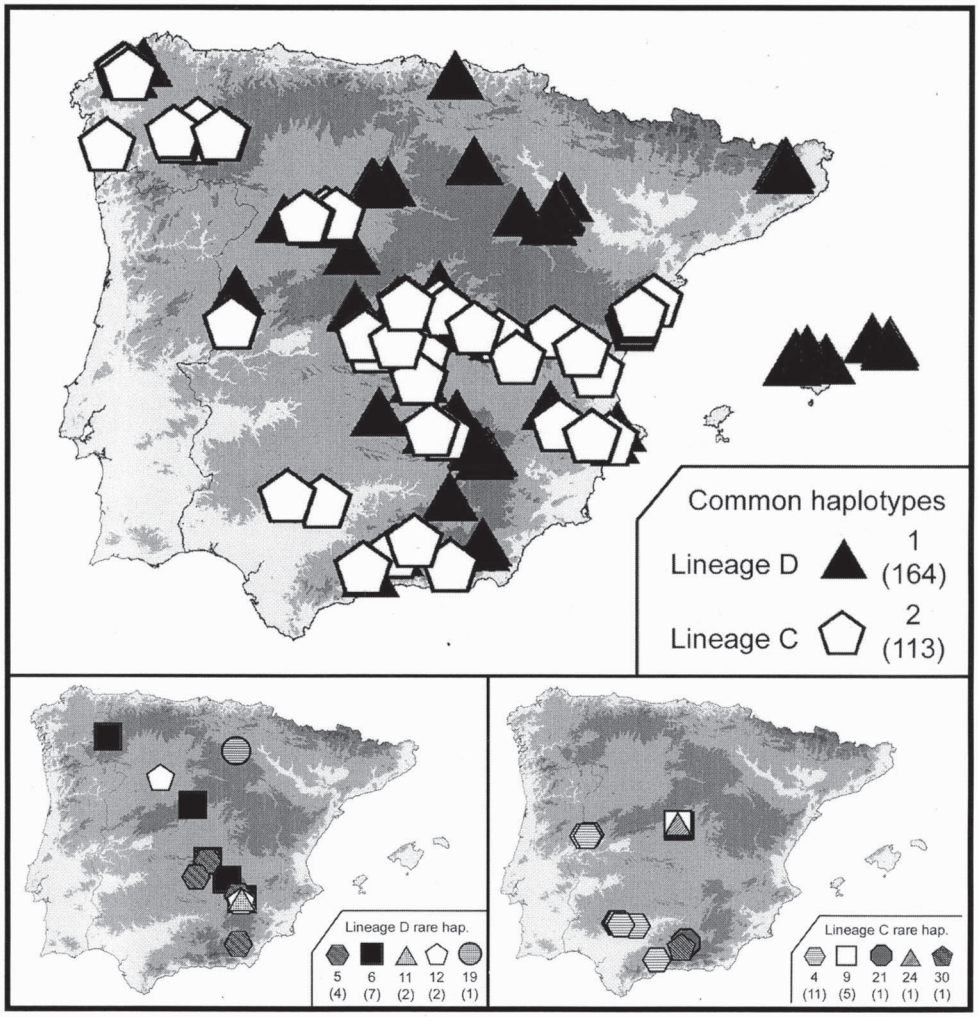

Figure 5. Distribution of cpDNA haplotypes in Spanish *Ulmus minor* and *U. glabra* samples. Lineages and numbers of haplotypes as described in Gil *et al.* (2004). Numbers in braquets indicate absolute frequencies of individuals considering both species altogether. All haplotypes were found in *U. minor. U. glabra* samples only showed haplotype 1 (48 individuals) and haplotype 12 (1 individual).

them very rare, in 49 samples from six distant populations). Populations of *U. glabra* are restricted to mountain regions where large-scale agriculture is hampered, thus it is likely that their cpDNA diversity was less influenced by human activities than in *U. minor* populations. If that is the case, the low diversity of lineage D in Spanish elms may be intrinsic to both tree species and prior to the Romans, and therefore we could conclude that total cpDNA variability was indeed increased thanks to elm cultivation.

However, a very different conclusion can be drawn if we analyse genetic diversity of nuclear microsatellites, as extracted from the data published by Collada *et al.* (2004). In the development of five nuclear microsatellite markers for *U. minor,* the authors analysed 30 samples of this species from six different Spanish populations (5 trees per population). The trees showed lineage C and lineage D cpDNA haplotypes (data not shown). They also tested cross-amplification in *U. glabra* in 29 trees, also from six Spanish populations (4-5 trees per population). Three microsatellites amplified in both species. Interestingly, the number of alleles per locus in *U. minor* samples (5, 4 and 8, respectively) was much lower than in *U. glabra* (8, 8 and 18). The lower nuclear variability of field elm populations is a consequence of the clonal

propagation of one genotype that corresponds to the Atinian elm which, following Collumela's recommendation, was widely planted by the Romans in the Iberian Peninsula (Gil *et al.,* 2004). The large distribution of this genotype, which botanists would later consider a variety of *U. minor* (var *vulgaris)*, reduced the genotypic diversity of elms in the Roman plantations. At the same time, the nuclear genetic diversity of native elm populations was also diminished not only after cuttings for cultivation in the areas where they grew, but also because the large number of copies of the Atinian elm increased the probability that local elms were pollinated and backcrossed by this genotype during centuries. This situation would explain why populations of *U. glabra,* which are rare and small, have preserved higher levels of nuclear diversity than *U. minor.* Female-sterility of the Atinian prevented it of being pollinated by local elms which, together with human propagation, preserved its genome unchanged.

When Dutch elm disease spread across Europe in the 20th century, *U. minor* var. *vulgaris* (the Atinian elm) turned out to be highly susceptible to the disease (Richens, 1983). As a consequence, the reduced genetic diversity of field elm in Spain caused by the abundance of the clone and continuous hybridisation with other genotypes reduced the chances of finding resistant genotypes in the populations (Gil and García Nieto, 1990), an unpredictable consequence of the process of domestication that this tree species suffered 2,000 years before.

Concluding remarks

Molecular markers have proved powerful tools to characterise the contrasting patterns of genetic diversity of Spanish forest tree populations. Moreover, by using complementary information such as palynological data or historical records of human management, we have been able to identify the main factors that affect the current level of genetic diversity of several of our main tree species. During the last ten years, we have been able to infer major issues in Spanish forests, such as domestication of field elm, identification of glacial refugia and postglacial recolonisation in oaks and pines, regional evolutionary dynamics of *P. sylvestris* or the value of island populations of trees as reservoirs of genetic diversity.

The complexity of Spanish forests is evident, since most of them presently evolve in secondary contact zones after recolonisation from multiple refugia, where trees endured glacial stages. On the other hand, we have shown that different species respond in different ways to perturbations. While *Ulmus minor* genetic diversity has been dramatically eroded by human intervention and the devastating effects of Dutch elm disease, the genetic dynamics of other species seems more resilient to environmental pressures, as is the case of *Pinus sylvestris* in the Northern Meseta, *Quercus pyrenaica* in the Sierra Norte, *Pinus canariensis* or the balearic evergreen oaks. The latter are examples of forest populations that maintain high levels of genetic diversity, even in marginal locations or under high levels of human intervention. Management and conservation policies should not disregard the valuable information obtained from molecular marker studies in order to preserve and make a rational use of our forests.

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References

ADLER G.H., 1992. Endemism in birds of tropical Pacific islands. Evol. Ecol. 6:296-306.

ALÍA R., NOTIVOL E., MORO J., 2001. Genetic variability of Spanish provenances of Scots pine *(Pinus sylvestris* L.): Growth traits and survival. Silva Fenn 35, 27-38.

ÁLVAREZ J.C., ALLUÉ M., 1997. Aspectos forestales en las Ordenanzas de la Comunidad de Villa y Tierra de Coca de 1583. Proceedings of the 2nd Spanish Forest Conference, Pamplona, pp. 383-388.

BOSSEMA I., 1979. Jays and oaks: an ecological study of symbiosis. Behaviour 70, 1-118.

BREHAUT E., 1933. Translator's note in Cato the Censor, In Farming (translated by E. Brehaut).. New York: Columbia University Press. 153 pp.

BREWER S., CHEDDADI R., DE BEAULIEU J.-L., REILLE M., DATA CONTRIBUTORS. 2002. The spread of deciduous *Quercus* throughout Europe since the last glacial period. For Ecol Manag 156, 27-48.

CARRIÓN J.S., YLL E.I., WALKER M.J., LEGAZ A.J., CHAÍN C., LÓPEZ A., 2003. Glacial refugia of temperate, Mediterranean and Ibero-North-African flora in south eastern Spain: new evidence from cave pollen at two Neanderthal man sites. Global Ecol Biogeogr 12: 119-129.

COLLADA C., FUENTES-UTRILLA P., GIL L., CERVERA M. T., 2004. Characterization of microsatellite loci in *Ulmus minor* Miller and cross-amplification in *U. glabra* Hudson and *U. laevis* Pall. Mol Ecol Notes 4, 731-732.

COLOM G., 1978. Biogeografía de las Baleares. La formación de las islas y el origen de su flora y de su fauna. Diputación Provincial de Baleares. Instituto de Estudios Baleáricos. CSIC. 515 pp.

COSTA TENORIO M., GARCÍA M., MORLA C., SAINZ H., 1990. La evolución de los bosques de la Península Ibérica: Una interpretación basada en datos paleobiogeográficos. Ecología, Fuera de Serie No. 1, 31-58.

CRONK Q.B.C., 1997. Islands: stability, diversity, conservation. Biodivers Cons 6(3), 477-493.

DE JONG H., 1998. In search of historical biogeographic patterns in the western Mediterranean terrestrial fauna. Biol J Linn Soc 65 (2), 99-164.

DEMESURE B., COMPS B. AND PETIT R. J., 1996. Chloroplast DNA phylogeography of the common beech *(Fagus sylvaticus* L.) in Europe. Evolution 50, 2515-2520.

DOW B.D., ASHLEY M.V., 1996. Microsatellite analysis of seed dispersal and parentage of saplings in bur oak, *Quercus macrocarpa.* Mol Ecol 5, 615-627.

DOW B.D., ASHLEY M.V., 1998. High levels of gene flow in bur oak revealed by paternity analysis using microsatellites. J Hered 89: 62-70.

DUMOLIN-LAPÈGUE S., DEMESURE B., LE CORRE V., FINESCHI S., PETIT RJ., 1997. Phylogeographic structure of white oaks throughout the European continent. Genetics 146, 1475-1487.

DUMOLIN-LAPÈGUE S., KREMER A., PETIT R.J., 1999. Are chloroplast and mitochondrial DNA variation species independent in oaks? Evolution 53, 1406-1413.

EL MOUSADIK A., PETIT R.J., 1996 Chloroplast DNA phylogeography of the argan tree of Morocco. Mol Ecol 5, *547-555.*

ENNOS R. A., 1994. Estimating the relative rates of pollen and seed migration among plant populations. Heredity 72, 250-259.

FERNÁNDEZ J., TORO M.A., CABALLERO A., 2003. Fixed contributions designs vs. minimization of global coancestry to control inbreeding in small populations. Genetics 165, 885-894.

FORSTER E. S., HEFFNER E. (trans.), 1954. Lucius Junius Moderatus Columella: On Agriculture II. Harvard University Press, Cambridge, Mass. Reprinted 1977. 503 pp.

FRANCO J., 1990. *Quercus.* En: Flora Ibérica. Plantas vasculares de la Península Ibérica e Islas Baleares Vol. II. (Castroviejo, S., Lainz, M., López-González, G., Montserrat, P., Muñoz Garmendia, F., Paiva J. y Villar, L. eds.). pp. 15-36. CSIC, Madrid.

FRANCO-MÚGICA F., GARCÍA-ANTÓN M., MALDONADO-RUIZ J., MORLA-JUARISTI C. SAINZ-OLLERO H., 2001. The Holocene history of *Pinus* forests in the Spanish Northern Meseta. Holocene 11, 343-358.

FRANCISCO-ORTEGA J., JANSEN R.K., SANTOS-GUERRA A., 1996. Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. P Natl Acad Sci USA 93, 4085-4090.

FUENTES-UTRILLA P., LÓPEZ-RODRÍGUEZ R. A., GIL L., 2004. The historical relationship of elms and vines. Invest. Agrar.: Sist. Recur. For. Vol 13(1), 7-15.

GATES D.M., 1993. Climate change and its biological consequences. Sinauer Associates INC, Sunderland, Massachusetts.

GIL L., GARCÍA-NIETO M.ª E., 1990. Paleobotánica e Historia de los olmos de la Península Ibérica. In: Los olmos y la grafiosis en España (Gil, L. ed.). ICONA. Madrid, pp. 29-65.

GIL L., PARDO F., ARANDA I., PARDOS J.A., 1999. El Hayedo de Montejo: Pasado y Presente. Comunidad de Madrid. 162 pp.

GIL L., CLIMENT J., NANOS N., MUTKE S., ORTIZ I., SCHILLER G., 2002. Cone morphology variation in *Pinus canariensis* Sm. Plant Syst Evol 235, 35-51.

GIL L., LÓPEZ R.A., GARCÍA-NIETO M.E., 2003. Historia de los olmos en la Península Ibérica. In: Los olmos ibéricos. Conservación y mejora frente a la grafiosis (L. Gil, A. Solla and S. Iglesias eds.). Madrid, pp. 69-114.

GIL L.; FUENTES-UTRILLA P.; SOTO A.; CERVERA M.T., COLLADA C., 2004. English elm is a 2,000-year-old Roman clone. Nature 431 (7012), 1053-1053.

GÓMEZ A., GONZÁLEZ-MARTÍNEZ S.C., COLLADA C., CLIMENT, J., GIL L., 2003. Complex population genetic structure in the endemic Canary Island pine revealed using chloroplast microsatellite markers. Theor Appl Genet 107, 1123-1131.

GÓMEZ A., VENDRAMIN G.G., GONZÁLEZ-MARTÍNEZ S.C., ALÍA R., 2005. Genetic diversity and differentiation of two Mediterranean pines *(Pinus halepensis* Mill. and *Pinus pinaster* Ait.) along a latitudinal cline using chloroplast microsatellite markers. Diversity Distrib 11, 257-263.

GÓMEZ A., LUNT D.H., 2005. Refugia Within Refugia: Patterns of phylogeographic concordance in the Iberian Penninsula. In: Phylogeography in Southern European Refugia: Evolutionary Perspectives on the origins and

conservation of European Biodiversity (S Weiss and N Ferrand, eds.). Kluwer Academic Publishers. Dordrecht, The Netherlands.

HAIG S., 1998. Molecular contributions to conservation. Ecology 79, 413-425.

HEWITT G.M., 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol J Linn Soc 58, 247-276.

HEYBROEK H.M., 2003. Los olmos en la historia y la cultura de centroeuropa. In: Los olmos ibéricos. Conservación y mejora frente a la grafiosis (L. Gil, A. Solla and S. Iglesias eds.) Madrid, pp. 19-43.

HOOPER W. D., ASH H. B. (trans.) Marcus Porcius Cato: On Agriculture. Marcus Terentius Varro: On Agriculture. Harvard University Press, Cambridge, Mass., 1934. Reprinted 1979. 542 pp.

HUNTER R., 1999. Theocritus. A selection: Idylls 1, 3, 4, 6, 7, 10, 11 and 13. Cambridge University Press. Cambridge, 308 pp.

HUNTLEY B., BIRKS H.J.B., 1983. An atlas of past and present maps for Europe: 0-13000 years ago. Cambridge University Press, Cambridge.

JOHNSON K.P., ADLER F.R., CHERRY J.L., 2000. Genetic and phylogenetic consequences of island biogeography. Evolution 54(2), 387-396.

C. SAINZ-OLLERO H., 2001. The Holocene history of *Pinus* forests in the Spanish Northern Meseta. Holocene 11, 343-358.

KENNINGTON W.J., JAMES S.H., 1997. The effect of small population size on the mating system of a rare clonal mallee, *Eucalyptus argutifolia* (Myrtaceae). Heredity 78, 252-260.

KLAUS W., 1989. Mediterranean pines and their history. Plant Syst. Evol. 162: 133-163.

KRIJGSMAN W., HILGEN F.J., RAFFI I., SIERRO F.J., WILSON, D.S., 1999. Chronology, causes and progression of the Messinian salinity crisis. Nature 400, 652-655.

LÓPEZ-DE-HEREDIA U., JIMÉNEZ P., DÍAZ-FERNÁNDEZ P., GIL L., 2005. The Balearic Islands: a reservoir of cpDNA genetic variation for evergreen oaks. J Biogeogr 32, 939-949.

MARIETTE S., COTTRELL J., CSAIKL U.M., GOICOECHEA P.G., KÖNIG A., LOWE A.J., *et al.,* 2002. Comparison of levels of genetic diversity detected with AFLP and microsatellite markers within and among mixed *Q. petraea* (Matt.) Liebl. and *Q. robur* L. stands. Silvae Genet 51: 72-79.

MARTIN R., 1971. Recherches sur les agronomes latins et leurs conceptions économiques et sociales. Les Belles Lettres, Paris. 415 pp.

MCCAULEY D.E., 1995. The use of chloroplast DNA polymorphism in studies of gene flow in plants. Trends EcolEvol 10, 198-202.

MONTERO G., CAÑELLAS I., 1999. Manual de reforestación y cultivo del alcornoque *(Quercus suber* L.). Ministerio de Agricultura, Pesca y Alimentación. INIA, Madrid. 103 pp.

MORGANTE M., FELICE N., VENDRAMIN G.G., 1998. Analysis of hypervariable chloroplast microsatellites in *Pinus halepensis* reveals a dramatic genetic bottleneck. In: Molecular tools for screening biodiversity (Karp A., Isaac P.G., Ingram D.S. eds.). Chapman & Hall, London, pp. 407-412.

MORITZ C., 1995. Uses of molecular phylogenies for conservation. Philos T Roy Soc B, 349:113-118.

NEWTON A.C., ALLNUTT T.R., GILLIES A.C.M., LOWE A.J., ENNOS R.A., 1999. Molecular phylogeography, intraspecific variation and the conservation of tree species. Trends Ecol Evol 4(14),140-145.

OLALDE M., HERRÁN A., ESPINEL S., GOICOECHEA P.G., 2002. White oaks phylogeography in the Iberian Peninsula. For Ecol Manag 156, 89-102.

OLINE D.K., MITTON J.B., GRANT M.C., 2000. Population and subspecific genetic differentiation in the Foxtail pine *(Pinus balfouriana)*. Evolution 54, 1813-1819.

PALMÉ A., SU Q., RAUTENBERG A., MANNI F., LASCOUX M., 2003. Postglacial recolonization and cpDNA variation of silver birch, *Betula pendula* Molec Ecol 12, 201-212.

PARSONS J.J., 1981. Human influence on the pine and laurel forests of the Canary Islands. Geogr Rev 71, 253-271.

PASCHKE M., ABS C., SCHMID B., 2002. Effects of population size and pollen diversity on reproductive success and offspring size in the narrow endemic *Cochlearia bavarica* (Brassicaceae). Am J Bot 89, 1250-1259.

PETIT R.J., PINEAU E., DEMESURE B., BACILIERI R., DUCOUSSO A., KREMER A., 1997.Chloroplast DNA footprints of postglacial recolonization by oaks. P Natl Acad Sci USA 94, 9996-10001.

PETIT R.J., CSAIKL U.M., BORDÁCS S., BURG K., COART E., COTTRELL J., *et al.,* 2002. Chloroplast DNA variation in European white oaks. Phylogeography and patterns of diversity based on data from over 2600 populations. For Ecol Manag 156, 5-26.

PRUS-GLOWACKI B., STEPHAN B.R., BUJAS E., ALÍA R., MARCINIAK A., 2003. Genetic differentiation of autochthonous populations of *Pinus sylvestris* (Pinaceae) from the Iberian Peninsula. Plant Syst Evol 239, 55-66.

RICHENS, R.H., 1983. Elm. Cambridge University Press, Cambridge, 347 pp.

RIVAS-MARTÍNEZ S., COSTA M., 1987. España insular I: Las Baleares. In: La vegetación de España (Peinado Lorca, M. y Rivas-Martínez, S. eds). Universidad de Alcalá de Henares. Alcalá de Henares: 487-513.

RIVAS-MARTÍNEZ S., COSTA M., SORIANO P., PÉREZ R., LLORENS L., ROSELLÓ J.A., 1992. Datos sobre el paisaje vegetal de Mallorca e Ibiza (Islas baleares, España). Itinera Geobotanica 6, 5-98.

ROBLEDO-ARNUNCIO J.J., SMOUSE P.E., GIL L., ALÍA R., 2004a. Pollen movement under alternative silvicultural practices in native populations of Scots pine *(Pinus*

sylvestris L.) in central Spain. For Ecol Manag 197, 245-255.

ROBLEDO-ARNUNCIO J.J., ALÍA R., GIL L., 2004B. Increased selfing and correlated paternity in a small population of a predominantly outcrossing conifer, *Pinus sylvestris.* Mol Ecol 13, 2567-2577.

ROBLEDO-ARNUNCIO J.J., COLLADA C., ALÍA R., GIL L., 2005. Genetic structure of montane isolates of *Pinus sylvestris* L. in a Mediterranean refugial area. J Biogeogr 32, 595-605.

SORANZO N., ALÍA R., PROVAN J., POWELL W., 2000. Patterns of variation at a mitochondrial sequence-tagged-site locus provides new insights into the postglacial history of European *Pinus sylvestris* populations. Mol Ecol 9, 1205-1211.

STREIFF R., DUCOUSSO A., LEXER C., STEINKELLNER H., GLOESSL J., KREMER A., 1999. Pollen dispersal inferred from paternity analysis in a mixed stand of *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. Mol Ecol 8, 831-841.

TABERLET P., FUMAGALLI L., WUST-SAUCY A.-G., COSSONS J.-F., 1998. Comparative phylogeography and postglacial colonisation routes in Europe. Mol Ecol 7, 453-464.

TERRADAS J., 2001. Ecología de la vegetación. Omega. Barcelona.703 pp.

THIRGOOD J.V., 1981. Man and the Mediterranean forest. Nueva york, Academic Press. 194 pp.

VALBUENA-CARABAÑA M., GONZÁLEZ-MARTÍNEZ S.C., SORK V.L., COLLADA C., SOTO A., GOICOECHEA P.G., GIL L. Gene flow and hybridisation in a mixed oak forest [*Quercus pyrenaica* Willd. and *Q. petraea* (Matts.) Liebl.] in central Spain. Heredity. *In Press.*

VAXEVANIDOU Z., GONZÁLEZ-MARTÍNEZ S.C., CLIMENT J., GIL L., 2004. Marginal populations of Canary Island pine *(Pinus canariensis)*: relationship to central areas and colonization patterns revealed by chloroplast microsatellites. Plant evolution in Mediterranean climate zones- IXth Meeting of the International Organization of Plant Biosystematics (IOPB), Valencia, Spain, May 2004.

VOGGENREITER V., 1976. Euphorbio canariensis - Pinetum canariensis ass. nov. y límite inferior del Pinar Canario en Gran Canaria. Vieraea 6, 3-16.

WILLIS K.J., BENNETT K.D., BIRKS H.J., 1998. The late Quaternary dynamics of pines in Europe. In: Ecology and biogeography of Pinus (.M. Richardson, ed.). Cambridge Univ. Press, New York, pp. 107-121.